

Biosafety Level 3

These questions are based on the Biosafety Level 3 section of *Biosafety in Microbiological and Biomedical Laboratories*, 3rd ed., pages 24-32.

Please circle the response that best describes the laboratory in which work with select agents will be carried out.

N.A. = not applicable. If you mark "N.A.", please provide a brief explanation below that item or on a separate page.

Standard Microbiological Practices

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| Yes, No, N.A. | 1. | Access to the laboratory is limited or restricted at the discretion of the laboratory director when experiments are in progress. |
| Yes, No, N.A. | 2. | Persons wash their hands after handling infectious materials and animals, after removing gloves, and when they leave the laboratory. |
| Yes, No, N.A. | 3. | Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the laboratory. Persons who wear contact lenses in laboratories should also wear goggles or a face shield. Food is stored outside the work area in cabinets or refrigerators designated for this purpose only. |
| Yes, No, N.A. | 4. | Mouth pipetting is prohibited; mechanical pipetting devices are used. |
| Yes, No, N.A. | 5. | All procedures are performed carefully to minimize the creation of aerosols. |
| Yes, No, N.A. | 6. | Work surfaces are decontaminated at least once a day and after any spill of viable material. |
| Yes, No, N.A. | 7. | All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method, such as autoclaving. Materials to be decontaminated outside of the immediate laboratory are to be placed in a durable, leakproof container and closed for transport from the laboratory. Materials to be decontaminated at off-site from the laboratory are packaged in accordance with applicable local, state, and federal regulations, before removal from the facility. |
| Yes, No, N.A. | 8. | An insect and rodent control program is in effect. |

Special Practices

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| Yes, No, N.A. | 1. | Laboratory doors are kept closed when experiments are in progress. |
| Yes, No, N.A. | 2. | The laboratory director controls access to the laboratory and restricts access to persons whose presence is required for program or support purposes. For example, persons who are immunocompromised or immunosuppressed may be at risk of acquiring infections. Persons who are at increased risk of acquiring infection or for whom infection may be unusually hazardous are not allowed in the laboratory or animal rooms. The director has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory. |
| Yes, No, N.A. | 3. | The laboratory director establishes policies and procedures whereby only persons who have been advised of the potential biohazard, who meet any specific entry requirements (e.g., immunization), and who comply with all entry and exit procedures, enter the laboratory or animal rooms. |
| Yes, No, N.A. | 4. | When infectious materials or infected animals are present in the laboratory or containment module, a hazard warning sign, incorporating the universal biohazard symbol, is posted on all laboratory and animal room access doors. The hazard warning sign identifies the agent, lists the name and telephone number of the laboratory director or other responsible person(s), and indicates any special requirements for entering the laboratory, such as the need for immunizations, respirators, or other personal protective |

measures.

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| Yes, No, N.A. | 5. | Laboratory personnel receive the appropriate immunizations or tests for the agents handled or potentially present in the laboratory (e.g., hepatitis B vaccine or TB skin testing). |
| Yes, No, N.A. | 6. | Baseline serum samples are collected and stored for all laboratory and other at-risk personnel. Additional serum specimens may be collected periodically, depending on the agents handled or the function of the laboratory. |
| Yes, No, N.A. | 7. | A biosafety manual is prepared or adopted. Personnel are advised of special hazards and are required to read and to follow instructions on practices and procedures. |
| Yes, No, N.A. | 8. | Laboratory personnel receive appropriate training on the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures. Personnel receive annual updates, or additional training as necessary for procedural changes. |
| Yes, No, N.A. | 9. | The laboratory director is responsible for insuring that, before working with organisms at Biosafety Level 3, all personnel demonstrate proficiency in standard microbiological practices and techniques, and in the practices and operations specific to the laboratory facility. This might include prior experience in handling human pathogens or cell cultures, or a specific training program provided by the laboratory director or other competent scientist proficient in safe microbiological practices and techniques. |
| Yes, No, N.A. | 10. | A high degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels. Needles and syringes or other sharp instruments should be restricted in the laboratory for use only when there is no alternative, such as parenteral injection, phlebotomy, or aspiration of fluids from laboratory animals and diaphragm bottles. Plasticware should be substituted for glassware whenever possible. |
| Yes, No, N.A. | a. | Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for injection or aspiration of infectious materials. Used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal; rather, they must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving. |
| Yes, No, N.A. | b. | Syringes which re-sheath the needle, needle-less systems, and other safe devices should be used when appropriate. |
| Yes, No, N.A. | c. | Broken glassware must not be handled directly by hand, but must be removed by mechanical means such as a brush and dustpan, tongs, or forceps. Containers of contaminated needles, sharp equipment, and broken glass should be decontaminated before disposal, according to any local, state, or federal regulations. |
| Yes, No, N.A. | 11. | All manipulations involving infectious materials are conducted in biological safety cabinets or other physical containment devices within the containment module. No work in open vessels is conducted on the open bench. |
| Yes, No, N.A. | 12. | Laboratory equipment and work surfaces should be decontaminated with an appropriate disinfectant on a routine basis, after work with infectious materials is finished, and especially after overt spills, splashes, or other contamination with infectious materials. Contaminated equipment should also be decontaminated before it is sent for repair or maintenance or package for transport in accordance with applicable local, state, or federal regulations, before removal from the facility. Plastic-backed paper toweling used on non-perforated work surfaces within biological safety cabinets facilitates clean-up. |
| Yes, No, N.A. | 13. | Cultures, tissues, or specimens of body fluids are placed in a container that prevents leakage during collection, handling, processing, storage, transport, or shipping. |
| Yes, No, N.A. | 14. | All potentially contaminated waste materials (e.g., gloves, lab coats, etc.) from laboratories or animal rooms are decontaminated before disposal or reuse. |
| Yes, No, N.A. | 15. | Spills of infectious materials are decontaminated, contained and cleaned up by appropriate professional staff, or others properly trained and equipped to work with concentrated infectious material. |
| Yes, No, N.A. | 16. | Spills and accidents which result in overt or potential exposures to infectious materials are immediately |

reported to the laboratory director. Appropriate medical evaluation, surveillance, and treatment are provided and written records are maintained.

- Yes, No, N.A. 17. Animals and plants not related to the work being conducted are not permitted in the laboratory.

Safety Equipment (Primary Barriers)

- Yes, No, N.A. 1. Properly maintained biological safety cabinets are used (Class II or III - see Appendix A) for all manipulation of infectious materials.
- Yes, No, N.A. 2. Outside of a BSC, appropriate combinations of personal protective equipment are used (e.g., special protective clothing, masks, gloves, face protection, or respirators), in combination with physical containment devices (e.g., centrifuge safety cups, sealed centrifuge rotors, or containment caging for animals).
- Yes, No, N.A. 3. This equipment must be used for manipulations of cultures and of those clinical or environmental materials which may be a source of infectious aerosols; the aerosol challenge of experimental animals; harvesting of tissues or fluids from infected animals and embryonated eggs, and necropsy of infected animals.
- Yes, No, N.A. 4. Face protection (goggles and mask, or face shield) is worn for manipulations of infectious materials outside of a biological safety cabinet.
- Yes, No, N.A. 5. Respiratory protection is worn when aerosols cannot be safely contained (i.e., outside of a biological safety cabinet), and in rooms containing infected animals.
- Yes, No, N.A. 6. Protective laboratory clothing such as solid-front or wrap-around gowns, scrub suits, or coveralls must be worn in, and not worn outside, the laboratory. Reusable laboratory clothing is to be decontaminated before being laundered.
- Yes, No, N.A. 7. Gloves must be worn when handling infected animals and when hands may contact infectious materials and contaminated surfaces or equipment. Disposable gloves should be discarded when contaminated, and never washed for reuse.

Laboratory Facilities (Secondary Barriers)

- Yes, No, N.A. 1. The laboratory is separated from areas which are open to unrestricted traffic flow within the building. Passage through two sets of self-closing doors is the basic requirement for entry into the laboratory from access corridors or other contiguous areas. A clothes change room (shower optional) may be included in the passage way.
- Yes, No, N.A. 2. Each laboratory contains a sink for hand washing. The sink is foot, elbow, or automatically operated and is located near the laboratory exit door.
- Yes, No, N.A. 3. The interior surfaces of walls, floors, and ceilings are water resistant so that they can be easily cleaned. Penetrations in these surfaces are sealed or capable of being sealed to facilitate decontamination.
- Yes, No, N.A. 4. Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.
- Yes, No, N.A. 5. Laboratory furniture is sturdy, and spaces between benches, cabinets, and equipment are accessible for cleaning.
- Yes, No, N.A. 6. Windows in the laboratory are closed and sealed.
- Yes, No, N.A. 7. A method for decontaminating all laboratory wastes is available, preferably within the laboratory (i.e., autoclave, chemical disinfection, incineration, or other approved decontamination method).
- Yes, No, N.A. 8. A ducted exhaust air ventilation system is provided. This system creates directional airflow that draws air from "clean" areas into the laboratory toward "contaminated" areas. The exhaust air is not recirculated to any other area of the building, and is discharged to the outside with filtration and other treatment optional. The outside exhaust must be dispersed away from occupied areas and air intakes. Laboratory personnel must verify that the direction of the airflow (into the laboratory) is proper.

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| Yes, No, N.A. | 9. | The High Efficiency Particulate Air (HEPA)-filtered exhaust air from Class II or Class III biological safety cabinets is discharged directly to the outside or through the building exhaust system. If the HEPA--filtered exhaust air from Class II or III biological safety cabinets is to be discharged to the outside through the building exhaust air system, it is connected to this system in a manner (e.g., thimble unit connection) ¹³⁶ that avoids any interference with the air balance of the cabinets or building exhaust system. Exhaust air from Class II biological safety cabinets may be recirculated within the laboratory if the cabinet is tested and certified at least every twelve months. |
| Yes, No, N.A. | 10. | Continuous flow centrifuges or other equipment that may produce aerosols are contained in devices that exhaust air through HEPA filters before discharge into the laboratory. |
| Yes, No, N.A. | 11. | Vacuum lines are protected with liquid disinfectant traps and HEPA filters, or their equivalent, which are routinely maintained and replaced as needed. |
| Yes, No, N.A. | 12. | An eyewash facility is readily available. |